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Hantavirus S RNA Sequence From a Fatal Case of HPS in New York

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In April, 1995, the second fatal case of hantavirus pulmonary syndrome (HPS) occurred in the northeast in a New York State resident. Using the patient's lung tissue obtained at autopsy, the S genomic RNA segment of a hantavirus, designated H-NY1, was amplified by reverse transcriptase-polymerase chain reaction (RT-PCR), cloned, and sequenced. The S RNA was found to contain 2084 nucleotides, 6 nucleotides longer than reported by Hjelle *et al.* (1995) for the virus associated with the first northeastern case (RI-1). There were 101 nucleotide differences in the S RNA between the H-NY1 and RI-1, which result in the prediction of a single amino-acid change in the nucleocapsid (N) protein. Rodents were trapped for serologic and virologic studies at the patient's residence and work site. The white-footed mouse (*Peromyscus leucopus*) was the most frequently captured species and more than 50% of those trapped near the patient's residence showed serologic evidence of hantavirus infection. Using RT-PCR it was possible to amplify hantavirus S RNA sequence from the lung tissues of 8 out of 11 seropositive animals. No difference in nucleotide sequence was found between the HPS patient sequence and the *P. leucopus* sequence (nucleotides 189 to 599). These data are consistent with those of Hjelle *et al.* (1995) in suggesting that *P. leucopus* is the primary rodent vector for the etiologic agent of HPS in the northeastern United States.

KEY WORDS: Hantavirus pulmonary syndrome, rodent, Bunyaviridae

INTRODUCTION

Hantaviruses are rodent-borne viruses belonging to the family Bunyaviridae. Their genomes consist of three negative, single-stranded RNA segments designated large (L), middle (M), and small (S), which encode the virus polymerase protein (L), two glycoproteins G1 and G2, and the nucleocapsid (N) protein, respectively (Elliott *et al.*, 1991). Recently, hantavirus pulmonary syndrome (HPS) was described in the southwestern United States (Nichol *et al.*, 1993; Duchin *et al.*, 1994; Hjelle *et al.*, 1994). HPS is caused by a novel hantavirus, *Sin nombre*, and like other known hantaviruses, is associated with a specific primary rodent vector (McKee *et al.*, 1991), in this case the deer mouse *Peromyscus maniculatus*. Several fatal cases caused by a rodent vector other than *P. maniculatus* were reported (Hjelle *et al.*, 1995; Rollin *et al.*, 1995; Khan *et al.*, 1995), including a fatal case of HPS in a Rhode Island/New York resident (Hjelle *et al.*, 1995). A second fatal case in the northeast occurred in a resident of Long Island, New York, in April, 1995. To determine the source of the patient's infection, we obtained, amplified, and sequenced viral S RNA from the patient and from rodents trapped on the grounds of the patient's residence.

MATERIALS AND METHODS

Case Report

In April 1995, a 25 year old previously healthy man died from an acute respiratory illness in Suffolk County, New York. The man had been well until three days earlier, when he experienced fever, chills, and lassitude. The day before he died, he went to a local emergency room and complained of fever, chills, and diarrhea. His temperature was 102.0 °F and his pulse was 114/minute. His physical examination was otherwise unremarkable. The next day the patient returned to the hospital in acute respiratory distress. His temperature was 94.5 °F and his respiratory rate was 40/min. His white blood cell count was 9,500/mL with 34% band forms, his hematocrit was 63.5%, and the platelet count was 69,000/mL. His $_{P}O_2$ was 49.0 mm Hg while breathing 100% O_2 (normal 80-100 mmHg) and the chest radiograph showed confluent bilateral pulmonary infiltrates. He was intubated and admitted to the intensive care unit but died five-hours later of respiratory failure.

The patient had not traveled outside of Long Island

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in the two months prior to his death. During the four weeks prior to his death, he worked for a landscaping company where his duties included weeding, planting, and clearing garden debris. Coworkers did not recall clearing rodent nests or seeing evidence of rodents at any of the work sites. The patient lived in a rodent-infested house adjoining a vacant lot. None of his immediate family or coworkers reported having a recent respiratory illness. Three family members and one coworker submitted blood for hantavirus serology and all were negative.

Trapping and Processing of Rodents

In May, 1995, small mammals were collected from the grounds of the patient's residence and from his place of employment following New York State Department of Health Animal Trapping protocols (1994). One hundred-thirty aluminum livetraps (H.B. Sherman Co., model #LFAHD) were baited with rolled oats for two nights and set inside and around the foundation of the residence, along the perimeter of other buildings on the property, and in the yard and wooded hedgerow surrounding the residence, within approximately 75 meters of the residence. An additional 70 traps were set at the patient's place of employment, a garden nursery located approximately eight miles from the residence. This resulted in a total of 400 trap nights at both locations. Traps were placed to obtain the highest rate of capture, based on apparent rodent activity, without regard to any particular spacing. All traps with animals were enclosed in individual plastic bags, placed in a large plastic bag, and transferred to a biosafety cabinet, where all subsequent steps were performed, including identification to species, weight, sex, and age, as well as surgical procedures to obtain blood and tissues.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

RT-PCR was conducted as described previously (Campbell and Huang, 1995). Briefly, approximately 100 mg of human lung tissue (or 40 mg of mouse lung) was transferred to a disposable tissue grinder, homogenized in 1.0 mL Ultraspec RNA (Biotecx Laboratories, Inc.), and RNA was extracted as described by the manufacturer. Random hexamers (BMB) were used to transcribe RNA (5%) to cDNA in a reverse-transcription reaction mixture (50 μL) containing 50 mM Tris-HCl, pH 8.3, 30 mM KCl, 8 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM of each dATP, dCTP, dGTP, and dTTP, plus 200 units of Moloney murine leukemia virus reverse transcriptase (BRL). It was incubated for 10 min at 20°C, 45 min at 37°C and 5 min at 99°C. An aliquot of the cDNA (5 μL) was then added to the PCR reaction mixture (50 μL), which consisted of 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 200 ng of two primers (HANTAS1: TGGACCCA-GATGACGTTAACAAAAG, nucleotides 143-167; HANTAS9: GCAAAAAATGCACCCAGTTCAGGCAT, nucleotides 1012-1037), 0.2 mM dNTP, and 2.5 units of Ampli-Taq DNA polymerase (Perkin-Elmer Cetus). Three to 4 μL of the PCR reaction mix was used as a template for the nested primer pairs, in a final volume of 50 μL. The thermal profile for PCR consisted of 25 cycles of 45 s at 94°C, 45 s at 52°C and 60 s at 72°C; followed by a 10-min polymerization step at 72°C to ensure that full-sized product was synthesized.

Serology

Human IgG and IgM antibody and rodent IgG antibody to hantavirus (Sin nombre) were determined by ELISA using procedures and reagents provided by the Special Pathogens Branch, US Centers for Disease Control and Prevention.

Sequence Analysis

The PCR product was loaded onto an agarose gel and the band with the expected size was excised, blunt-ended using T_4 DNA polymerase (Bio Lab) and ligated into the Hinc II site of pUC18. The plasmid with the cDNA insert was used to transform Escherishia coli DH5α. Transformants were screened for S RNA-specific inserts as described by Maniatis *et al.* (1982). Nucleotide sequences were determined using the dideoxynucleotide method (Sanger *et al.,* 1977), either on denatured double-stranded plasmid DNA or on PCR product using a Sequenase Version 2.0 sequencing kit (United States Biochemical Corp.). Sequence data were analyzed using the program devised by the University of Wisconsin Genetic Computer Group (UWGCG) (Devereux *et al.,* 1984).

RESULTS

Cloning of the S RNA genome of H-NY1

Numerous pairs of the S RNA segment primer designed using published and unpublished sequences (Parrington and Kang, 1990; Spiropoulou *et al.,* 1994, Hjelle, personal communication) were used in PCR reactions. One-fourth of the first PCR reaction was loaded onto an agarose gel containing ethidium bromide and the bands with the expected size were visible (data not shown). The complete sequence of the S RNA of H-NY1 consists of 2084 nucleotides (GenBank accession number U47135), 6 nucleotides longer than that of RI-1 (Hjelle *et al.,* 1995). Although the H-NY1 differed from the RI-1 by 4.8% at the nucleotide level, there is only one predicted amino acid change in the N protein. Of the 50 nucleotide differences in the coding region of the N protein, 47 (94%) were transitions, primarily A to G or T to C, and 86% of the base substitutions occurred at codon position 3.

The S segment of H-NY1 encodes a 428 amino acid nucleocapsid protein. There is a second, overlapping ORF (192 nucleotides) with a coding potential of 63 amino acids. Phylogenetic analysis has shown that the RI-1 is distinct from hantaviruses found in other parts of the country (Hjelle *et al.,* 1995). However, the two northeastern strains, H-NY1 and RI-1, are almost identical at the nucleotide and amino-acid levels and appear to represent the first members of another distinct group of hantavirus variants associated with HPS.

TABLE I. Serologic and RT-PCR Results for the Rodents Captured

Location[a]	Species	No. captured	*Sin nombre* ELISA IgG	RT-PCR
Residence	*P. leucopus* (white-footed mouse)	17	11	8
	M. pennsylvanicus (meadow vole)	3	1	1
Work site	*P. leucopus* (white-footed mouse)	3	0	0
	B. brevicauda (short-tailed shrew)	1	0	0
	Total	24	12	9

[a]Refers to the HPS patient in Suffolk County, New York, 1995.

RT-PCR of Seropositive *Peromyscus leucopus*

Twenty-four small rodents were trapped from two locations in Suffolk County, NY; the patient's residence and from a landscaping company where he worked. As expected, *Peromyscus leucopus* (white-footed mouse) was the most abundant species captured (20 of 24; 83.3%, Table 1). RNA extraction and RT-PCR analysis were performed on lung tissue from all 24 rodents. Visible PCR bands on agarose gel were obtained from 8 of 11 seropositive *P. leucopus* in the first-round PCR, but were absent in the negative controls (Table 1). Rodents trapped at the patient's work site were negative in both ELISA and RT-PCR assays. The nucleotide sequence of the PCR band amplified from lung tissue of a rodent (lab no. 9523010) captured near the patient's home was determined to be identical to that of H-NY1.

One of the seropositive rodents, *Microtus pennsylvanicus* (meadow vole), is the primary rodent reservoir for another hantavirus, Prospect Hill virus. Sequence data for the S RNA from lung tissue of the New York vole indicated that it is, as expected, a Prospect Hill-like virus (to be reported elsewhere).

DISCUSSION

There have been two cases of HPS recognized so far in the northeastern United States. The first occurred in 1994 in a Rhode Island College student who had visited New York City and Long Island, New York, shortly before becoming ill. Follow-up to that case resulted in hantavirus sequences being recovered from *P. leucopus* trapped on Long Island that were extremely similar to those of the virus detected in tissue from the patient (Song *et al.*, 1994). This, and the failure to recover any seropositive mice from sites in Rhode Island frequented by the patient, led investigators to believe that the infection was contracted in New York (CDC, 1994). This paper summarizes findings from the 1995 case of HPS and the subsequent investigation. The data are again compatible with the hypothesis that the infection occurred on Long Island. Although the cases were investigated independently by different laboratories, all of the sequence data are consistent in indicating that another hantavirus variant capable of producing HPS exists in the northeast and that the white-footed mouse, *P. leucopus,* is its primary rodent reservoir.

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REFERENCES

Campbell WP, Huang C (1995): Detection of California serogroup viruses using universal primers and reverse transcription-polymerase chain reaction. Journal of Virological Methods 53:55–61.

Centers for Disease Control and Prevention (1994): Hantavirus pulmonary syndrome—Northeastern United States, Morbidity and Mortality Weekly Report 43:549, 555–556.

Devereux J, Haeberli P, Smithies O (1984): A comprehensive set of sequence analysis program for the VAX. Nucleic Acids Research 12:387–395.

Duchin JS, Koster FT, Peters CJ, Simpson GL, Tempest B, Zaki SR, Ksiazek TG, Rollin PE, Nichol S, Umland ET, Moolenaar RL, Reef SE, Nolte KB, Gallaher MM, Butler JC, Breiman RF, the Hantavirus Study Group (1994): Hantavirus pulmonary syndrome: A clinical description of 17 patients with a newly recognized disease. New England Journal of Medicine 330:949–955.

Elliott RM, Schmaljohn CS, Collett MS (1991): Bunyavirus genome structure and gene expression. Current Topics in Microbiology and Immunology 169:91–141.

Hjelle B, Jenison S, Torrez-Martinez N, Yamada T, Nolte K, Zumwalt R, MacInnes K, Myers G (1994): A novel hantavirus associated with an outbreak of fatal respiratory disease in the southwestern United States: Evolutionary relationship to known hantaviruses. Journal of Virology 68:592–596.

Hjelle B, Krolikowski J, Torrez-Martinez N, Chavez-Giles F, Vanner C, Laposata E (1995): Phylogenetically distinct hantavirus implicated in a case of hantavirus pulmonary syndrome in the northeastern United States. Journal of Medical Virology 46:21–27.

Khan AS, Spiropoulou CF, Morzunov S, Zaki SR, Kohn MA, Nawas SR, McFarland L, Nichol ST (1995): Fatal illness associated with a new hantavirus in Louisiana. Journal of Medical Virology 46: 281–286.

Maniatis T, Fritsch EF, Sambrook J (1982): Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, N. Y.

McKee KT Jr, LeDuc JW, Peters CJ (1991): Hantavirus. In Belshe RB (ed): "Textbook of Human Virology." 2nd ed. St. Louis: Mosby Year Book, pp 615–632.

New York State Department of Health Animal Trapping Protocols. NYSDOH, 1994.

Nichol ST, Spiropoulou CF, Morzunov S, Rollin PE, Ksiazek TG, Feldmann H, Sanchez A, Childs J, Zaki S, Peters CJ (1993): Genetic identification of a novel hantavirus associated with an outbreak of acute respiratory illness in the southwestern United States. Science 262:914–917.

Parrington MA, Kang CY (1990): Nucleotide sequence analysis of the S genomic segment of Prospect Hill virus: comparison with the prototype Hantavirus. Virology 175:167–175.

Rollin PE, Ksiazek TG, Elliott LH, Ravkov EV, Martin ML, Morzunov S, Livingstone W, Monroe M, Glass G, Ruo S, Khan AS, Childs JE, Nichol ST, Peters CJ (1995): Isolation of black Creek Canal virus, a new hantavirus from *Sigmodon hispidus* in Florida. Journal of Medical Virology 46:35–39.

Sanger F, Nicklen S, Coulson AR (1977): DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences U.S.A. 74:5463–5467.

Song JW, Baek LJ, Gajdusek DC, Yanagihara R, Gavrilovskaya I, Luft BJ, Mackow ER, Hjelle B (1994): Isolation of pathogenic hantavirus from white-footed mouse (*Peromyscus leucopus*) Lancet 344:1637.

Spiropoulou CF, Morzunov S, Feldmann H, Sanchez A, Peters CJ, Nichol ST (1994): Genome structure and variability of a virus causing hantavirus pulmonary syndrome. Virology 200:715–723.